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L1: Entry 1 of 1 File: USPT Sep 7, 1999

US-PAT-NO: 5948682
DOCUMENT-IDENTIFIER: US 5948682 A

TITLE: Preparation of heterologous proteins on oil bodies

DATE-ISSUED: September 7, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Moloney; Maurice M.	Calgary			CA

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Sembiosys Genetics Inc.	Calgary			CA	03

APPL-NO: 08/ 846021 [PALM]
DATE FILED: April 25, 1997

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS The present application is a continuation-in-part of U.S. Ser. No. 08/366,783 that was filed on Dec. 30, 1994, now U.S. Pat. No. 5,650,554, which is a continuation-in-part of U.S. Ser. No. 08/142,418 that was filed Nov. 16, 1993, now abandoned, which is a continuation-in-part of U.S. Ser. No. 07/659,835 that was filed on Feb. 22, 1991, now abandoned.

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FIELD-OF-SEARCH: 435/69.1, 435/69.2, 435/69.52, 435/69.6, 435/69.7, 435/69.8, 435/70.1, 435/71.1, 435/172.3, 435/183, 435/214, 435/219, 435/254.2, 435/254.21, 536/23.2, 536/23.4, 536/23.52, 536/23.6, 536/24.1

PRIOR-ART-DISCLOSED:

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
0193259	September 1986	EP	

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ART-UNIT: 169

PRIMARY-EXAMINER: Fox; David T.

ATTY-AGENT-FIRM: Bereskin & Parr

ABSTRACT:

The present invention relates to the use of a class of genes called oil body protein genes that have unique features. The discovery of these features allowed the invention of methods for the production of recombinant proteins wherein a protein of interest can be easily separated from other host cell components. The invention is further exemplified by methods for exploitation of the unique characteristics of the oil body proteins and oil body genes for expression of polypeptides of interest in many organisms, particularly plant seeds. Said polypeptides may include but are not limited to: seed storage proteins, enzymes, bioactive peptides, antibodies and the like. The invention can also be modified to recover recombinant polypeptides fused to oil body proteins from non-plant host cells. Additionally the invention provides a method of using recombinant proteins associated with seed oil bodies released during seed germination for expression of polypeptides that afford protection to seedlings from pathogens. Finally, the persistent association of oil body proteins with the oil body can be further utilized to develop a biological means to create novel immobilized enzymes useful for bioconversion of substrates.

20 Claims, 8 Drawing figures

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CLAIMS:

What I claim as my invention is:

1. A method for the expression of a heterologous polypeptide by a yeast host cell said method comprising:

a) introducing into the yeast host cell a chimeric DNA sequence comprising:

1) a first DNA sequence capable of regulating the transcription in said yeast host cell of

2) a second DNA sequence, wherein said second sequence encodes a fusion polypeptide and comprises (i) a DNA sequence encoding a sufficient portion of an oil body protein gene to provide targeting of the fusion polypeptide to a lipid phase linked in reading frame to (ii) a DNA sequence encoding the heterologous polypeptide; and

3) a third DNA sequence encoding a termination region functional in the yeast host cell; and

b) growing said yeast host cell to produce the fusion polypeptide.

2. The method according to claim 1 further including separating the recombinant fusion polypeptide from cellular host cell components by selective partitioning into a lipid phase.

3. The method according to claim 2 wherein said selective partitioning comprises centrifugation, floatation or size exclusion.

4. The method according to claim 1 further including separating the recombinant

fusion polypeptide from cellular host components by selective partitioning into a lipid phase comprising oil bodies.

5. The method according to claim 4 wherein said recombinant fusion polypeptide is separated by addition of oil body components and reconstitution of the oil bodies.

6. The method according to claim 2 further comprising releasing the heterologous polypeptide from the fusion polypeptide associated with the lipid phase, said method comprising:

c) including in said second DNA sequence (2) between said DNA sequence (i) encoding the oil body protein and the DNA sequence (ii) encoding the heterologous polypeptide, a linker DNA sequence (iii) encoding an amino acid sequence that is specifically cleavable by enzymatic or chemical means; and

d) contacting the lipid phase with said enzymatic or chemical means such that said heterologous polypeptide is released from the fusion polypeptide.

7. The method according to claim 6 wherein said linker DNA sequence encodes an amino acid sequence that is recognizable by the proteolytic action of an enzyme selected from the group consisting of thrombin, factor Xa, collagenase, chymosin, clostrapain and viral protease.

8. The method according to claim 6 wherein said enzymatic means comprises an enzyme that is immobilized.

9. The method according to claim 8 wherein said enzyme is immobilized by attachment to an oil body protein that is associated with an oil body.

10. The method according to claim 1 wherein said recombinant polypeptide is an enzyme.

11. The method according to claim 10 wherein said recombinant polypeptide is an enzyme that retains its enzymatic properties while part of the fusion polypeptide is associated with the oil body.

12. The method according to claim 1 wherein said heterologous polypeptide is selected from the group consisting of antibodies, glycanases, hormones, proteases, protease inhibitors and seed storage proteins.

13. The method according to claim 1 wherein said heterologous polypeptide is selected from the group consisting of a thrombin inhibitor, hirudin, an interleuken, chymosin, cystatin, xylanase, carp growth hormone, zein, an antibody and a collagenase.

14. A method for the expression of a heterologous polypeptide by a yeast host cell said method comprising:

a) generating by homologous recombination into the yeast host cell a chimeric DNA sequence comprising:

1) a first DNA sequence capable of regulating transcription in said yeast host cell;

2) a second DNA sequence, wherein said second sequence encodes a fusion polypeptide and comprises (i) a DNA sequence encoding a sufficient amount of an oil body protein gene to provide targeting of the fusion polypeptide to a lipid phase, linked in reading frame, to (ii) a DNA sequence encoding the heterologous polypeptide; and

3) a third DNA sequence encoding a termination region functional in said yeast

host cell; and

b) growing said yeast host cell to produce the heterologous polypeptide.

15. A chimeric DNA sequence, capable of being expressed in association with an oil body of a yeast host cell, comprising:

1) a first DNA sequence capable of regulating the transcription in said yeast host cell of

2) a second DNA sequence, wherein said second sequence encodes a fusion polypeptide and comprises (i) a DNA sequence encoding a sufficient portion of an oil body protein gene to provide targeting of the fusion polypeptide to a lipid phase linked in reading frame to (ii) a DNA sequence encoding the heterologous polypeptide; and

3) a third DNA sequence encoding a termination region functional in the yeast host cell.

16. The chimeric DNA sequence according to claim 15 wherein said DNA sequence (ii) encodes an enzyme.

17. The chimeric DNA sequence according to claim 15 further including (iii) a linker DNA sequence encoding an amino acid sequence that is specifically cleavable by enzymatic means wherein said linker DNA sequence (iii) is located between said (i) DNA sequence encoding the oil body protein and said (ii) DNA sequence encoding the heterologous polypeptide.

18. The chimeric DNA according to claim 17 wherein said linker DNA sequence (iii) encodes a cleavage site for an enzyme selected from the group consisting of thrombin, factor Xa, collagenase chymosin and viral protease.

19. An expression cassette comprising a chimeric DNA sequence according to claim 15.

20. The method according to claim 1 or claim 14 wherein said yeast cell is Saccharomyces cerevisiae.